PATENT
242/027

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of: **Neil H. Bander** **Serial No.:** 08/895,914 **Filed:** July 17, 1997 **For:** TREATMENT AND DIAGNOSIS OF CANCER	**Group Art Unit:** 1642 **Examiner:** Yvonne Eyler

DECLARATION OF NEIL H. BANDER UNDER 37 CFR § 1.132

Box CPA
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, NEIL H. BANDER, pursuant to 37 C.F.R. § 1.132, declare as follows:

1. I hold a B.A. degree in Biology from Johns Hopkins University and a M.D. degree from the University of Connecticut Medical School.

2. I am a Professor of Urology, Weill Medical College of Cornell University where I am also the Bernard and Josephine Chaus Chair in Urologic Oncology and Surgical Director of Urologic Oncology.

3. I am an Attending Surgeon, Department of Urology, New York Presbyterian Hospital-Cornell University Medical Center.

CERTIFICATE OF MAILING
(37 C.F.R. §1.10)

I hereby certify that this paper (along with any referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as 'Express Mail Post Office To Addressee' in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

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Signature of Person Mailing Paper

4. I am also an Assistant Member at Memorial Sloan-Kettering Cancer Center, a Clinical Assistant Surgeon in the Urology Service and Clinical Immunology Service at Memorial Hospital, a Research Associate at Sloan-Kettering Institute for Cancer Research, and an Assistant Member of the Ludwig Institute for Cancer Research at Memorial Sloan-Kettering Cancer Center.

5. As explained more fully in my attached *Curriculum vitae,* I have authored numerous publications in the field of Urologic Oncology. **(Exhibit A.)**

6. I am the sole inventor of the above-identified patent application.

7. I have reviewed the Office Action mailed from the United States Patent Office on January 21, 1999 regarding the above-identified patent application. In this paper, the Examiner states at page 4 that "the specification does not provide sufficient guidance and evidence to enable one of skill in the art to predictably obtain agents or antibodies that bind to the extracellular domain of PSMA and target tumor vasculature." To the contrary, the procedures for producing the antibodies of my invention are fully described in the application. In particular, the four antibodies described in the application (i.e. E99, J415, J533 and J591) were prepared as set forth in Example 3. In fact, I used this same approach more than once to generate the four different antibodies. Antibody E99 is derived from a completely different immunization/fusion experiment than the remaining antibodies denoted by the "J" prefix, i.e. J415, J533 and J591.

8. In addition, at page 5 of the January 21, 1999 Office Action, the Examiner states that "the specification provides evidence of the ability of the antibodies to target to tissues *in vitro* for detection but provides insufficient objective evidence that antibodies to PSMA extracellular domain or the antibodies E99, J415, J533 and J591 effectively target to tumor vasculature *in vivo*." The Examiner cites Jain, R.K. (Cancer and Metastasis Reviews, 9:753-266,

1990) as teaching "that the efficacy in cancer treatment of novel therapeutic agents such as monoclonal antibodies, cytokines and effectors cells has been limited by their inability to reach their target *in vivo* in adequate quantities." Several physiological factors "responsible for the poor localization" are set forth by the Examiner including "(I) heterogeneous blood supply, (ii) elevated interstitial pressure which lowers fluid extravasation, and (iii) large transport distances in the interstitium." As I detail below, these factors are not relevant in the approach or clinical setting of my invention and I provide additional *in vitro* and *in vivo* data as evidence that the antibodies of my invention do effectively target both prostate tumors and non-prostate tumor vasculature.

9. The factors recited by the Examiner are not relevant to the use of the antibodies of my invention for the detection and treatment of prostate cancer. First, metastatic prostate cancer predominantly involves the bone marrow and lymph nodes. These sites are, by definition, highly vascularized. In addition, prostate cancer is unlike many other solid tumors in that its metastatic sites are small volume sites measured in microns or millimeters. For this reason, as well as because of their presence in bone marrow and lymph nodes, these sites are very well supplied with antibody given the high levels of circulating antibody in the vascular compartment. Large transport distances, as a result, are not operative in this situation. Similarly, interstitial pressure in these small volume sites within bone marrow and lymph node is also not operative. Moreover, primary prostate cancers are also relatively small and multifocal and the factors recited by the Examiner are likewise not relevant.

10. With respect to targeting the PSMA molecule on vascular endothelial cells with the antibodies of my invention, the factors cited by the Examiner similarly are not operative. Since blood supply is necessary to allow tumor nutrition and growth, and since we are directly

targeting blood vessels, the heterogeneity of blood supply is not relevant. Since we do not require antibody to extravasate into the tumor in the setting in which we are targeting the vasculature itself, elevated interstitial pressure is not relevant. And, of course, since again we are targeting the blood vessels themselves and do not require antibody extravasation, the issues of large transport distances also is not relevant.

11. In addition to data presented in the application, further *in vitro* data demonstrating effective targeting (as well as ablation) of tumor cells using the antibodies of my invention is presented in Yang et al., AACR Abstract #2996 (1998) and a poster presentation presented by Ballangrud et al., attached as **Exhibit B.** These data are generated using LNCaP spheroids which are LNCaP cells that grow *in vitro* as tumor masses several hundreds of microns in diameter, rather than in a monolayer. These spheroid masses recapitulate an *in vivo* tumor mass to an extent and demonstrate the ability of the antibodies of my invention to penetrate into tumor masses. Indeed, these spheroids are substantially larger than the typical prostate cancer metastasis. As shown in the data, the antibodies of my invention conjugated with a florescein marker or isotope, are observed by confocal microscopy to penetrate into these tumor masses. Beyond this penetration, use of the radioisotope antibody conjugate, shows that these relatively large tumor spheroids can be effectively destroyed or killed. Also included in Exhibit B is a series of graphs and photos which examine the volume of multiple spheroids over time, treated with and without dexamethasone and/or ^{90}Ytrium labeled specific (J591) or nonspecific (HuM195) antibody.

12. In addition to the *in vitro* data mentioned above, *in vivo* animal data which demonstrates the ability of the antibodies of my invention to target tumor sites is appended in **Exhibit C.** These 2 graphs examine my antibodies J591 and J415, as well as 7E11, conjugated

to two different isotopes and demonstrate by quantitative analysis, that there is selective and specific uptake of radiolabeled antibody by PSMA–expressing tissues in an animal model (i.e. tumor xenografts). More specifically, these graphs demonstrate that, over the 6-8 day period of observation, the relative amount of antibody in the tumor as compared to either blood or muscle continues to increase. Not shown on these graphs is that an irrelevant antibody (B1) showed significantly lower tumor to non-tumor ratios than those found with these antibodies specific for PSMA.

13. Clinical *in vivo* data demonstrating targeting of the antibodies of my invention to a non-prostate cancer in a human patient is appended as **Exhibit D**. This patient **[#6]** had both hormone-refractory prostate cancer and biopsy-proven colon cancer that had spread (metastasized) to the liver. The two photographs represent the patient's CAT scan and the patient's antibody scan. The CAT scan shows sequential slices through the liver demonstrating the mass in the right lobe of the liver. As shown in the antibody scan of this patient following administration of a radiolabeled antibody of my invention, there is intense uptake of radiolabel and therefore significant signal in the vasculature of the same liver metastasis, indicating antibody localization to this non-prostate cancer.

14. The Examiner also notes at page 6 of the Office Action that "the demonstration of *in vitro* binding to tissue samples provides insufficient objective evidence that the instant antibody-toxins are predictively effective in ablating or killing cancer cells in the *in vivo* clinical situation based on *in vitro* binding to cells," and that "there is no indication that binding of the J591 antibody to live LNCaP cell[s] had any effect on their viability." As noted above in paragraphs 11 and 12, the antibodies of my invention have been shown to target tumor cells *in vivo* in both an animal model and the clinical setting. Moreover, in paragraph 10, *in vitro* data

using LNCaP spheroids demonstrates the ability of the antibodies of my invention to kill LNCaP cells growing in a tumor mass.

15. Data from an additional *in vitro* experiment appended in **Exhibit E** demonstrate that the J591 antibody (both humanized and mouse) mediates antibody dependent cellular cytotoxicity (ADCC). That is, human lymphocytes and anti-PSMA antibody will induce a lysis of human prostate cancer cells. Controls in the studies consist of no antibody and no effector cells, and humanized and mouse versions of an anti-leukemia ("irrelevant") antibody plus cells. This is yet another mechanism by which the antibodies of the present invention demonstrate their cytotoxicity. And as explained below, we have shown the antibodies of my invention are effective in killing tumor cells *in vivo* in both an animal model and the clinical setting.

16. The data appended in **Exhibit F** shows that the use of a radiolabeled (^{213}Bismuth) antibody of my invention can delay and/or prevent tumor growth *in vivo* in an animal model. In this animal study, the animals were inoculated with a human prostate xenograft of LNCaP cells and several days later were treated with ^{213}Bismuth conjugated J591 antibody. Two control groups were studied. One group received no treatment whatsoever and the second group received ^{213}Bismuth conjugated to an irrelevant antibody which targets human leukemia cells but not prostate cancer cells. There were 6 animals per treatment group. Figure 2 is the serum PSA (a prostate cancer antigen detectable in the serum of individuals with prostate cancer and an indicator of the presence/progression of prostate cancer) data of the mice shown in Figure 1. In brief, the data shows that on day 51, the PSA levels in these mice is substantially higher in the two control groups than in the groups given anti-PSMA antibody conjugate. This study demonstrates that there was a significant delay in the development of tumor growth in the anti-PSMA treated animals and half of those animals never developed detectable tumors.

17. The first results of our recent experiments using antibody J591 conjugate in mice having large LNCaP xenograft tumors of approximately 1 cm in diameter, (this represents 5% of the animal's body weight) have shown similar xenograft killing effects. **Exhibit G** includes data from a number of different studies. Firstly, data labeled "G1", are results of a large series of animals treated with ^{131}Iodine-muJ591 (mouse J591) at different doses (100mCi or 300 mCi) and different routes of administration (intraperitoneal and intravenous). The animals in these studies got a single treatment dose on day 0 approximately 10-14 days past tumor implantation, when tumors had reached approximately 1 cm in diameter. Control animals received a single injection. Each line represents a growth curve for an individual tumor. Exhibit "G2a" shows a point to point tracing of the average size of tumors in a group of animals (3-5 animals per group) treated with saline (PBS), J591 alone, J591 conjugated to a cytotoxin or animals treated with J591 conjugated to a different cytotoxin. The same data appears in exhibit "G2b" except that the curves are now "fitted" by a computer program. "G2b" also indicates the number of animals in each group. Exhibit "G2c" shows a plot of the weight of the animals in the different treatment groups showing that there was not significant adverse effect on the animals weight due to the treatment. However, the control (PBS) animals suffered the most with respect to weight as they became increasingly cachectic due to the increasing size of their tumors. Exhibit "G3a" and "G3b" are graphs which show that the cytotoxic conjugates can effectively kill LNCaP cells *in vitro*.

18. The data appended in **Exhibit H** show that the use of radiolabeled antibodies and "naked" antibodies of my invention in a human patient are effective at both imaging/localization to non-prostate cancer and resulting in a measurable shrinkage of tumor. More specifically, this patient received humanized J591 antibody. The first 6 doses of antibody included a combination

of "naked" antibody and antibody labeled with a trace amount of I^{131} which was for diagnostic purposes only and not intended as a therapeutic dose. Thereafter, this patient received 3 doses of purely "naked" antibody. The four photographs in Exhibit H are from the CAT scan and indicate nodal involvement in the neck, mediastinum, retroperitoneum and retrocrural area and pelvis. An additional photo is the patient's bone scan. Also included is a PET scan which demonstrates uptake of radiolabeled antibody in most of the areas shown on the CAT scan. The photograph labeled "H1" is the planar scan of the patient's antibody study which demonstrates uptake in the left neck node, mediastinum and retroperitoneum as well as pelvic nodes. This can also be seen on the SPECT study labeled "H2" where uptake in the neck node, mediastinum as well as the right shoulder (consistent with the increased uptake in the right shoulder on the bone scan). This patient has, to date, had a 25% shrinkage of his measurable left neck mass and a 50% decline in his PSA.

19. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

DATE: 7/19/99

NEIL H. BANDER